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EXAMINER	
MEAH, MOHAMMAD Y	

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/587,769	SARIBAS ET AL.	
	Examiner	Art Unit	
	MD. YOUNUS MEAH	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 January 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5,7-11,14,31 and 32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5,7-11,14,31 and 32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

With supplemental amendment, filed 1/25/2010, in response to the office action, mailed on 8/04/2009, the applicants cancel claims 2, 3, amended claims 1, 11, 14 and added new claims 31, 32. Claims 1, 5, 7-11, 14 and 31-32 are currently pending in the instant application. Claims 1, 5, 7-11, 14 and 31-32 will be examined.

Applicants' arguments filed on 1/25/2010 have been fully considered but they are found unpersuasive. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections 35 U.S.C 103a

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all Obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 7, 10-11, 14 remain rejected and claim 31 is rejected under 35 U.S.C. 103(a) as being unpatentable over Paulson et al (US patent 5858751) in view of Hellman et al (protein expr, pur. 1995, 6, 56-62, from IDS) and Clark et al (Current opin. Biotech. 2001, 202-207, from IDS). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claims 1 and 14 are directed to a method of refolding insoluble eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) protein by solubilizing in a solubilizing buffer and

refolding using a refolding buffer comprising a redox couple wherein the refolded ST3Gal3 catalyzes the transfer of sialic acid sugar from a donor to an acceptor substrate. Claim 31 is directed to a method to refold an insoluble eukaryotic ST3GAl3 which lacks part of or all of its stem region wherein said method requires solubilizing the insoluble eukaryotic protein using a refolding buffer comprising a redox couple. Claim 7 is directed to the method of claim 1 wherein said eukaryotic ST3Gal3 is expressed in a bacterial host cell as an insoluble inclusion body. Claim 10 is directed to the method of claim 1 wherein said eukaryotic ST3Gal3 is refolded by using GSH/GSSG. Claim 11 is directed to the method of claim 1 wherein said acceptor is a protein, peptide glycoprotein or glycopeptide. acid.

Paulson et al. teach multiple methods and expression of various eukaryotic alpha (2, 3) sialyltransfereases (ST3Gal3) in eukaryotic and prokaryotic organisms (whole document) including expression of mutated proteins particularly derivatives lacking the stem region (column 75, claim 4) and teach the transferring of sialic acid from a CMP-sialic acid donor (column 7 lines 15-20) to a glycoprotein (column 4 lines 22-30). Paulson is silent regarding maltose binding domain and the refolding of the alpha (2, 3) sialyltransferease (ST3Gal3) using a buffer comprising a redox system.

Clark et al teach a method of isolation, purification and refolding of insoluble proteins from inclusion bodies (page 202, column 2) using a disulfide bond forming redox buffer such as GSH/GSSG (page 205, column 1). Clark does not teach refolding alpha (2, 3) sialyltransferease (ST3Gal3) nor teach fusion protein comprising maltose binding domain.

Hellman et al teach solubilizing an insoluble protein from inclusion bodies by expression of an N-terminal fusion of the desired protein with maltose binding domain (MBD) (such as, MBD fusion of CGT(cyclomaltodextrin glucanotransferase), see page 60, column 2).

It would have been obvious to one of ordinary skill in the art to combine the teachings of Paulson et al, Hellman et al and Clark et al to produce a biologically active soluble eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) by expressing in a prokaryotic organism a fusion protein consisting of ST3Gal3 and the well known purification MBD tag, solubilize the inclusion bodies and refold using a refolding buffer comprising reduced glutathione/oxidized glutathione (GSH/GSSG).

One of ordinary skill in the art would have been motivated to do so because eukaryotic alpha (2, 3) sialyltransfereases (ST3Gal3) are used in the production of other glycosylated proteins and/or specific oligosaccharides that are useful as pharmaceuticals for the treatment of various disorders (Paulson et al column 2 lines 38-68). One of ordinary skill in the art would have been also motivated to express the eukaryotic alpha (2, 3) sialyltransferease in prokaryotic system because i) use of prokaryotes in recombinant production proteins is well known in the art, ii) prokaryotic system is commercially available (cheaper to produce), iii) It is easier to purify recombinant proteins of interest from prokaryotes, iv) prokaryotic systems are potentially free of eukaryotic pathogens. Maltose binding domain (MBD) is a very well known as a purification tag (Hellman et al.). One of skill in the art would have been

motivated to express the eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) as a fusion protein comprising MBD in a prokaryotic system.

Therefore a skilled artisan would be motivated to employ well known prokaryotic recombinant expression system to produce a fusion protein comprising the alpha(2, 3) sialyltransferease (ST3Gal3) and the MBD and to use the method of Clark et al. to refold the said fusion protein to produce alpha (2, 3) sialyltransferease (ST3Gal3) on a large scale and apply the method of Hellman et al (applying MBD fusion) and Clark et al to refold (using redox buffer such as GSH/GSSG) the said protein in an active form so that it catalyzes the transfer of sialic acid sugar from a CMP-sialic acid to galactose containing substrate. There is a reasonable expectation of success in view of the fact that Clark teach that the recited method can be applied to any insoluble protein, the use of MBD fusion proteins is well known in the art as evidenced by Hellman et al and the recombinant production of proteins in prokaryotic cells is well known and widely practiced in the art. Therefore, the claimed method as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made

Arguments and response

Applicants' argue, at pages 6-14 of their amendment of 1/25/2010, that the one of ordinary skill in the art although would have had a reason to modify the teachings of the cited art to recombinantly produce eukaryotic α–(2,3) sialyltransferase (ST3Gal3) of Paulson et al using methods of Clark and Hellman, s/he would not have had reasonable expectation of success of obtaining a solubilized and refolded ST3Gal3 protein comprising a maltose binding protein starting from an insoluble protein because

production of solubilized and refolded ST3Gal3 protein comprising a maltose binding protein would not have been predictable. Applicants argue that none of the three references teach or suggest obtaining soluble active ST3Gal3 by refolding insoluble protein from inclusion body they only teach "general approach".

Applicants' arguments filed on 1/25/2010 have been fully considered, but they found unpersuasive. There is nothing unpredictable or unexpected from obtaining an active enzyme purified from inclusion bodies. Many proteins and enzymes have been over expressed and purified from inclusion bodies (Hellman *et al*). It is well known in prior art , how to produce biologically active soluble eukaryotic proteins by expressing in a prokaryotic organism by a fusion of the protein with the MBD tag, solubilize from the inclusion bodies and refold using refolding buffer, see Hellman *et al,* Bach et al (J. Mol. Biol. 2001, 312, pp 79-93, from IDS); Kapust et al (Protein Sci 199, 8, pp 1668-1674, from IDS). Applicants' argument that none of the references disclose obtaining soluble active ST3Gal3 by refolding insoluble protein from inclusion body is true, if they do, they would anticipate applicants' invention. However, as indicated above, the three references provide not only the individual elements of the claimed method but also the motivation to combine these elements as well as a reasonable expectation of success at obtaining a soluble active eukaryotic ST3Gal3 by over expressing said eukaryotic ST3Gal3 as MBP fusion protein in a prokaryotic organism, solubilizing the inclusion under denaturing conditions, and refolding the fusion protein in appropriate buffer comprising GSH/GSSG for proper formation of disulfide linkages.

Applicant further argue that Paulson et al produce recombinant eukaryotic sialyltransferases using eukaryotic expression systems and did not use prokaryotic expression systems. Although Paulson et al. did not provide a working example, the reference still teaches expression in prokaryotic systems (column 10 lines 37-46) and there is no reason to believe that such teaching is not enabled in view of the fact that production of eukaryotic proteins in prokaryotic systems is well known (Clark et al, introduction section) and widely practiced in the art. Applicant further argue that Hellman et al teach solubilizing insoluble cyclomaltodextrin glucanotransferase protein from inclusion bodies by expression of N-terminal fusion of desired protein with maltose binding domain (MBD) and which is different from alpha (2,3) sialyltransferase (ST3Gal3) recited in applicants' present claims. However, Hellman et al teaching of solubilizing insoluble cyclomaltodextrin glucanotransferase from inclusion bodies by expressing MBD fusion of said protein could be used for solubilizing alpha (2,3) sialyltransferase (ST3Gal3) from inclusion bodies by N-terminal fusion of the protein with maltose binding domain (MBD).

Applicant further argue that Kapust teach that TEV protease is solubilized by MBP fusion not ST3GaLIII. Applicants' argument is considered but found unpersuasive. As explained above not only Kapust et al but also many other references teach solubilizing biologically active soluble eukaryotic proteins by expressing in a prokaryotic organism by a fusion of the protein with the MBD tag, solubilize from the inclusion bodies, see Hellman *et al,* Bach et al (J. Mol. Biol. 2001, 312, pp 79-93, from IDS). Applicant use the cited reference of Nomine et al (Protein expression and purification,

2001, 23, 22-32) to illustrate the point that most soluble MBP-E6 protein is misfolded. Applicant also use the cited reference of Boeggeman et al (Protein expression and purification, 2003, 33, 219-229) to illustrate the point that most expressed proteins such as, galactosyltransferase, in *E. coli* are misfolded. Applicants' argument is considered but found unpersuasive. A MBP fusion protein is used to solubilize the protein not for refolding and a refolding buffer comprising reduced glutathione/oxidized glutathione (GSH/GSSG) is used to refold the protein (as discussed above in the 103(a) rejection above).

Therefore, as it was discussed above, the use of maltose binding domain to solubilize and purify insoluble proteins from inclusion bodies of prokaryotic expression systems is well known in prior art (Hellman et al, Bach et al, J. Mol. Biol. 2001, 312, pp 79-93, from IDS; Kapust et al Protein Sci 199, 8, pp 1668-1674, from IDS)., Also well known in the art is the refolding of folded protein using a disulfide bond forming redox buffer such as GSH/GSSG (Clark et al). Therefore one of ordinary skill in the art would combine the teachings of Paulson et al., Hellman et al and Clark et al to produce a biologically active soluble eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) by expressing in a prokaryotic organism a fusion protein consisting of ST3Gal3 and the well known purification MBD tag, solubilize the inclusion bodies and refold using a refolding buffer comprising reduced glutathione/oxidized glutathione (GSH/GSSG).

Applicant further argue that their invention comprises unexpected results. Applicants have provided no evidence or a reasonable scientific argument to support

surprising or unexpected results. Thus, the claimed invention remains *prima facie* obvious over the prior art of record.

Claim 32 is rejected under 35 U.S.C. 103(a) as being unpatentable over Paulson et al (US patent 5858751) in view of Hellman et al (protein expr, pur. 1995, 6, 56-62, from IDS) and Clark et al (Current opin. Biotech. 2001, 202-207, from IDS) and further in view of Ramakrishnan et al (J. Biol. Chem. 2001, 276, 37665-37671). This is a new rejection necessitated by amendment..

The teachings of Paulson et al., Hellman et al and Clark et al are summarized above.

Ramakrishnan et al teach that mutation of an unpaired cysteine, Cys342 to Thr of a beta-galacotosyltransferase resulted in 2 to 3 fold increase in yield of refolded enzyme (compare to unmutated enzyme, page 37666, 1st column last paragraph)).

It would have been further obvious to one of ordinary skill in the art to combine the teachings of Paulson et al., Hellman et al and Clark et al with the teachings of Ramakrishnan et al to produce a biologically active soluble eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) in high yield by expressing in a prokaryotic organism a fusion protein comprising an ST3Gal3 protein which has been mutated such that the unpaired cysteine residues have been substituted with non-cysteine residues (as taught Ramakrishnan et al) and the well known MBD tag, solubilize from the inclusion bodies and refold using refolding buffer in order to get better yield of refolded protein.

Arguments and response

Applicants' argument, 6-14 of their amendment of 1/25/2010, against claim 32 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for the Claims 1, 7, 10-11 and 14. Applicants' further argue that Ramakrishnan et al worked with a different protein and that there is no way to reasonably predict a successful outcome using ST3Gal3. Applicants' argument have been fully considered, but they found unpersuasive. Examiner acknowledges that the reference uses a different protein but that a minimum, one of skill in the art would have been motivated to make the substitution in view of the successful results of Ramakrishnan and that there is a reasonable expectation of success at substituting the cysteine residues because the molecular biology techniques required are well known in the art. Applicants' further argue that they obtained unexpected results and that one of skill in the art would not have been able to reasonably predict the outcome. Applicants' argument have been fully considered, but they found unpersuasive. Applicants' method as claimed requires not just making the substitution of the cysteine residues for refolding but also exposing the protein to a refolding buffer comprising a redox couple. As long as there is a reasonable expectation of success at refolding the recited protein by using a refolding buffer containing a redox couple, that is all that is required by the claims. Whether the substitution of the cysteine residues increases the refolding yield or not is irrelevant because there is no limitation requiring any particular level of refolding or any particular yield.

Claim 5 remains rejected under 35 U.S.C. 103(a) as being unpatentable over Paulson et al (US patent 5858751) in view of Hellman et al (protein expr, pur. 1995, 6,

56-62, from IDS), Clark et al Current opin. Biotech. 2001, 202-207, from IDS) and further in view of Nilsson et al. (Protein expression and purification 1997, 11, pp 1-16, IDS). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claim 5 is directed to the method of claim 1 wherein said eukaryotic ST3Gal3 further comprise purification domain selected from the group consisting of a starch binding domain, a thioredoxin domain, and poly-his domain.

The teachings of Paulson et al., Hellman et al and Clark et al are summarized above.

It is well known in prior art that a convenient method of purification of protein is make a fusion protein wherein target protein is fused with one or more affinity tags (such as one or more affinity tags from the group of maltose binding domain, starch binding domain, a thioredoxin domain, glutathione-S-transferase (GST) domain and poly-HIS domain, Nilsson et al.). Nilsson et al. teach the use of two affinity tags attached to target protein (table 2, GST and Poly-HIS domains, page 5, 1st column 2nd pargh.) and purify the target protein using two affinity columns (Fig 4 at page 8).

It would have been further obvious to one of ordinary skill in the art to combine the teachings of Paulson et al., Hellman et al and Clark et al to produce a biologically active soluble eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) by expressing in prokaryotic organism a fusion protein consisting of ST3Gal3 and the well known purification MBD tag with the teachings of Nilsson et al. to add a second purification tag such as, poly-HIS domain, solubilize said protein having MBD tag and the 2nd

purification tag from the inclusion bodies and refold using refolding buffer and purify by using two affinity columns so that said purified ST3Gal3 catalyzes the transfer of sialic acid sugar from a CMP-sialic acid to galactose containing substrate.

Arguments and response

Applicants' argument, 6-14 of their amendment of 1/25/2010, against claim 5] have been fully considered, but they found unpersuasive, as explained above in the response against the argument for the Claims 1-2, 7, 10-11 and 14. Applicants' further argue that Nilsson et al. did not work or suggest fusion of MBD with ST3Gal3. Applicants' argument have been fully considered, but they found unpersuasive. Examiner acknowledges that the reference uses a different protein but that a minimum, one of skill in the art would have been motivated to add an additional purification domain to the fusion protein comprising MBD and ST3Gal3 in view of the successful results of Nilsson et al in the purification of proteins using two or more affinity tags and that there is a reasonable expectation of success at adding an additional purification domain would facilitate the purification using affinity chromatography. Furthermore, there is no evidence that having two purification tags would prevent the refolding of the protein in the method of Paulson, Hellman and Clark, and the method as claimed requires other steps for refolding in addition to the presence of two tags. As long as there is a reasonable expectation of success at refolding by using the buffer containing the redox couple, one of skill in the art would have a reasonable expectation of success with regard to the method of Paulson, Hellman, Clark and Nilsson.

Claims 8-9 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Paulson et al (US patent 5858751) in view of Hellman et al (protein expr, pur. 1995, 6, 56-62, from IDS) and Clark et al Current opin. Biotech. 2001, 202-207, from IDS). This rejection is maintained as discussed at length in the previous office action and discussed it again.

Claims 8-9 are directed to the method of claim 1 wherein one or two additional recombinant eukaryotic glycosyltransferases is (are) refolded with said eukaryotic ST3Gal3.

The teachings of Paulson et al., Hellman et al and Clark et al are summarized above.

It is well known in prior art that most bioactive glycoproteins comprise variety of sugar residues and need multienzymes to produce them (see Paulson et al column 4 lines 21- 44). It would have been further obvious to one of ordinary skill in the art, for the production of a multienzyme system to catalyze the transfer (in addition to sialic acid sugar) of sugar moieties from donors to acceptor substrate, to combine the teachings of Paulson et al., Hellman et al and Clark et al to produce biologically active soluble eukaryotic alpha (2, 3) sialyltransferease (ST3Gal3) by expressing in prokaryotic organism a fusion protein consisting ST3Gal3 and the well known MBD purification tag, solubilize from the inclusion bodies and further refold 2 or more glycosyltransferases with it using refolding buffer so that said multienzyme system catalyzes the transfer (in addition to sialic acid sugar) other sugar moieties from donor to acceptor substrate. A skilled artisan would be motivated to solubilize and refold one

or two additional recombinant eukaryotic glycosyltransferases with said eukaryotic ST3Gal3 because refolding more enzymes at the same time would save time and reagents. There is a reasonable expectation of success in view of the fact that Clark teach that the recited method can be applied to any insoluble protein, the use of MBD fusion proteins is well known in the art as evidenced by Hellman et al and the recombinant production of proteins in prokaryotic cells is well known and widely practiced in the art. Therefore, the claimed method as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made

Arguments and response

Applicants' argument, 6-14 of their amendment of 1/25/2010, against claim 8 and 9 have been fully considered, but they found unpersuasive, as explained above in the response against the argument for the Claims 1-2, 7, 10-11 and 14.

Allowable Subject Matter/Conclusion

None of the claims are allowable.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mohammad Meah whose telephone number is 571-272-1261. The examiner can normally be reached on 8:30-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

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Mohammad Younus Meah
Examiner, Art Unit 1652

/Delia M. Ramirez/

Primary Examiner, Art Unit 1652